The Succinonitrile Triple-Point Standard: A Fixed Point to Improve the Accuracy of Temperature Measurements in the Clinical Laboratory

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In an investigation of the melting and freezing behavior of succinonitrile, the triple-point temperature was determined to be 58.0805 °C, with an estimated uncertainty of ±0.0015 °C relative to the International Practical Temperature Scale of 1968 (IPTS-68). The triple-point temperature of this material is evaluated as a temperature-fixed point, and some clinical laboratory applications of this fixed point are proposed. In conjunction with the gallium and ice points, the availability of succinonitrile permits thermistor thermometers to be calibrated accurately and easily on the IPTS-68.

Additional Keyphrases: *quality control · instrument calibration · analytical error*

Because temperature is such an important factor in many tests conducted in the clinical laboratory, accurate temperature measurements and control are essential for improving the precision and accuracy of those tests and for making comparisons of test results meaningful, both within the same laboratory from day to day and among laboratories. For accurate temperature measurements, thermometers must be calibrated, and this is done, either directly or indirectly, through the use of temperature-fixed points (*1*). Because temperature-fixed points provide reproducible environments, they are ideally suited for, and have been used in, defining practical temperature scales [the latest version is the International Practical Temperature Scale of 1968 (IPTS-68), amended edition of 1975 (*2, 3*)] and for calibration of thermometers on those scales. If a sufficient number of temperature-fixed points are available in the temperature range of interest, thermometers may be calibrated directly through use of those fixed points; otherwise, they must be calibrated by comparison with another thermometer, the accuracy of which must be approximately an order of magnitude greater than that desired for the thermometers to be calibrated. The latter procedure is common for thermometers used in clinical laboratories, there being currently only two conveniently usable temperature-fixed points in the temperature range of biomedical interest: the gallium melting point[1] (*4, 5*) at 29.772 °C and the ice point of water at 0 °C. Although these two fixed points are not sufficient in number to permit calibrations, they provide the means to check a thermometer's calibration.

Here I present data on another excellent temperature-fixed point for the clinical laboratory, the triple point of succinonitrile [SCN; $NC(CH_2)_2CN$],[2] and point out some of its potential applications.

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[1] Cells of gallium, Standard Reference Material 1968 (the Gallium Melting-Point Standard), are available from the National Bureau of Standards.

[2] Nonstandard abbreviations: SCN, succinonitrile; SRM, Standard Reference Material; SPRT, Standard Platinum Resistance Thermometer.

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Materials and Methods

Succinonitrile Samples

The samples of SCN were zone-refined material[3] that was hermetically sealed in small borosilicate glass cells. Figure 1 is a drawing, with dimensions, of a cell containing approximately 60 g of SCN. The cells were designed to provide sufficient immersion for thermistor thermometers in the cells' thermometer wells. Moreover, the cells hold enough SCN for the liquid–solid equilibrium to be maintained for several hours when they are in an environment at a temperature within several tenths of a degree Celsius of the melting/freezing point of SCN. Some pertinent physical properties of SCN are given in Table 1.

Each small cell contains approximately 60 g of SCN, which is estimated to be 99.999% to 99.9999% pure, assuming Raoult's law of dilute solutions (*10, 11*).[4] Although samples taken from different sections of the zone-refining tube were found to have slight differences in purity, the first two or three samples obtained from the purest portion of the zone-refined material had temperatures at the plateau of the melting/freezing curves that differed by no more than 0.001 or 0.002 °C.

[3] All samples were purchased from Prof. M. Glicksman of Rensselaer Polytechnic Institute, Troy, NY.

[4] A confirmation of these purities by another technique, although highly desirable, would be very difficult and expensive in testing for unknown impurities in organic materials of this purity. Also, the tests would necessarily result in destruction of the triple-point cells holding the samples.

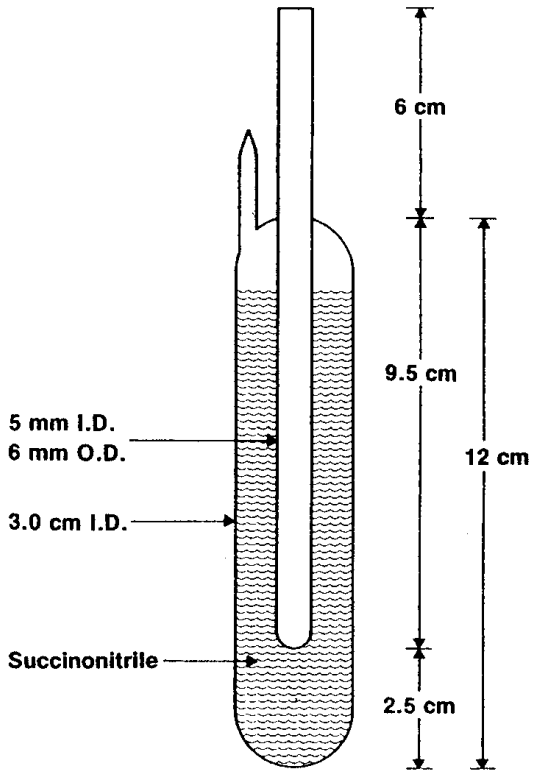

Fig. 1. Drawing of small succinonitrile cell used in this investigation

Table 1. Properties of Succinonitrile

Property	Value	Reference
Rel. molecular mass	80.092	6
Density of solid	1.016 g/cm^3 [a]	6
Density of liquid	0.988 g/cm^3 [a]	7
Thermal expansity of solid	-5.6×10^{-4}/K	8
Thermal expansity of liquid	-8.1×10^{-4}/K	7
Latent heat of fusion	46.24 J/g	6
Thermal conductivity of solid	2.25×10^{-3} W/cm · K	9
Thermal conductivity of liquid	2.23×10^{-3} W/cm · K	9

[a] At the melting point.

The results reported here are for six samples of SCN, designated SCN-1, SCN-4, SCN-5, SCN-201, SCN-202, and SCN-203. Samples SCN-201, SCN-202, and SCN-203 were taken successively from the same zone-refining tube, with SCN-201 being obtained from the highest purity part of the material. Similarly, SCN-4 and SCN-5 were obtained from the highest purity segment of SCN in another zone-refining tube, SCN-4 being the first segment. SCN-1 was obtained from material that remained after filling a large cell (*12*).

Thermometry

Bead-in-glass probe-type thermistor thermometers (*13, 14*), which have resistances of approximately 3400 Ω at the liquid–solid transition temperature of SCN, were used in this investigation. These thermometers were calibrated at the triple point of water, the melting point of gallium, and 13 other temperatures over the range from 0 to 70 °C against an SPRT in a copper comparison block situated in an oil bath maintained at the selected constant temperatures (constant to within about ±0.0002 °C). A Cutkosky a.c. resistance bridge (*15*) was used in making measurements with the SPRT.

Melting and Freezing Experiments

Melting and freezing experiments on the SCN samples involved several different temperature gradients, the difference between the bath temperature and the melting/freezing temperature of SCN ranging from about 0.3 to 2.3 °C in the different experiments. In the melting experiments, a given sample at a temperature of about 22 °C was placed in a temperature-controlled oil bath maintained at a selected temperature, which was higher than the liquidus temperature of SCN. The temperature in the thermometer well was measured with the thermistor thermometer at regular intervals from the time the cell was placed in the bath until the sample was completely melted.

In preparation for freezing experiments, the samples were completely melted by immersing the cells in a bath at approximately 65 °C. Then, one of two methods was used for the freezing process. In one method, the *inner-sheath* method, a cold copper rod was inserted into the thermometer well and left there until solid SCN could be seen forming around the well. Then the rod was withdrawn, the thermometer inserted, and the assembly placed in an oil bath maintained at a selected constant temperature that was lower than the solidus temperature of SCN. The temperature in the thermometer well was then monitored until the sample had totally solidified. For the second type of freezing experiments, the *outer-sheath* method, the thermistor thermometer was placed in a cell containing liquid SCN at a temperature of about 65 °C, and the assembly was placed directly in the temperature-controlled oil bath at the desired temperature. The temperature in the thermometer well was monitored as the SCN solidified from the outside wall of the cell inward.

Results

SCN is a material (*12*) in which the deleterious effects of the properties of real materials on the realization of its liquid–solid transition temperature can be minimized and thus its liquid–solid equilibrium temperature can be well defined. The behavior of ideal materials during their transitions from the solid to the liquid phase and from the liquid to the solid phase, and some of the reasons the behavior of real materials departs from that of ideal materials, are well known (*16, 17*). Among the properties of real materials that affect their melting and freezing behavior are finite thermal conductivity, the mechanisms of melting and freezing, effects of pressure, and effects of impurities. Pressure effects due to changes in atmospheric pressure during melting or freezing of SCN have been avoided by using hermetically sealed cells containing SCN at its triple-point temperature. Strains (or internal pressures), however, may be present in a material and normally could cause broadening of the liquid–solid transition, but for SCN the effects of this should be negligible (*12*).

Typical melting and freezing curves of SCN are shown in Figure 2. The inner-sheath method (the preferred technique) was used in obtaining the freezing curve. As shown, the liquid–solid equilibrium temperature may be attained either by melting or by freezing, and once it has been attained, it is available for a long time for calibration of thermometers. There is rounding of the melting curve at the beginning and ending of the solid–liquid transition because of the finite thermal conductivity of SCN. The melting curve in Figure 2 shows that as the SCN melted from the outside of the cell inward toward the thermometer well, there was only a slight increase in temperature, a total change of about 0.001 °C. This was ascribable to the presence of impurities. The part of the curve referred to as the plateau can be considered as being in two segments. The first part represents the liquid–solid equilibrium, or near-equilibrium, and it occurred from about the 60th to the 330th minute (for the particular sample and conditions involved). Beginning at about the 330th minute, some change in the environment of the thermometer occurred, causing the temperature of the thermometer to increase by about 0.0005 °C over a period of several minutes. This was caused by some physical change in the SCN mantle, although the actual details of that change are not known. A liquid–solid interface may have formed along some parts of the thermowell, or the thermometer may have started sensing the outer SCN liquid through some small channel created along grain boundaries. It is unlikely, however, that the

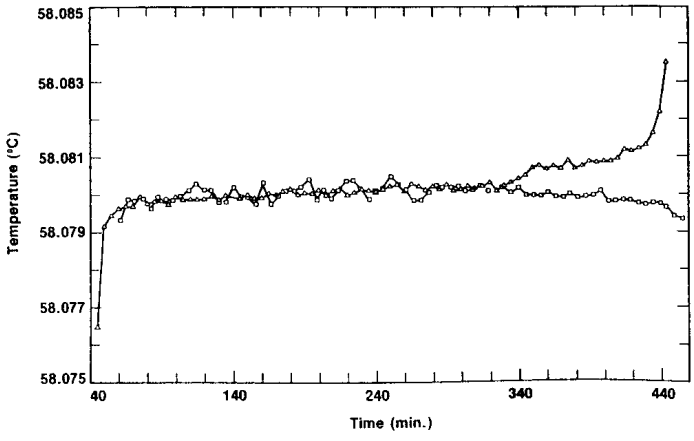

Fig. 2. Melting and freezing behavior of succinonitrile cell SCN-201
Δ represents data obtained on melting (bath at 58.37 °C), □ represents data obtained on freezing (bath at 57.79 °C)

SCN mantle slid down along the thermometer well, because in similar experiments in which we visually observed the melting process, the mantle remained firmly attached to the thermometer well until the SCN melted downward from the top of the cell to the bottom of the thermometer well. In some experiments, the rise in temperature owing to this phenomenon, whatever its cause, occurred earlier in the melt (e.g., see the curve for SCN-203 of Figure 4). After the change occurred in the curve of Figure 2, the melting process continued until approximately the 440th minute, at which time all of the solid had melted and the temperature of the liquid began its exponential approach to the bath temperature.

The initial part of the freezing curve shown in Figure 2 cannot be compared with an ideal curve, because the SCN freeze was initiated by inserting a cold copper rod into the thermometer well. Beyond that point, however, the curve is fairly close to that for an ideal material having finite thermal conduction and containing a small amount of impurities.

As illustrated in Figure 3, freezing curves of different samples, taken either from the same zone-refining tube or from different tubes, show the effects of impurities, with the most impure samples having plateaus with the lowest temperatures.

The melting behavior of SCN-1, SCN-4, SCN-5, SCN-201, and SCN-203 are compared in Figure 4. Because SCN-1 was material remaining after filling a large cell (*12*), we considered it to be the most impure of all the samples discussed here, and that opinion was confirmed by its melting behavior. Its triple-point temperature is only about 0.001 or 0.002 °C lower than that of SCN-4 and SCN-5, but its melting range, about 0.004 °C, is considerably larger. In spite of the relatively poor quality of SCN-1, however, it is still useful as a practical temperature reference point. The behaviors of samples SCN-4 and SCN-5 are essentially the same, at least to within about 0.001 °C. Of the two, SCN-4 has a somewhat wider melting range, indicating greater impurity. One would have expected just the opposite, because SCN-4 was the first portion of the zone-refined material. The glass sample holders in which the refined material was placed finally were washed with the zone-refined material at times during the refining process, so it is possible that some contaminant was not removed and thus is responsible for the observed behavior. The melting curves of SCN-201 and SCN-203 show the effects of increasing impurity content of samples taken from successive parts of the zone-refined material, with SCN-201 being the purer. The purity of SCN-202 should be intermediate to that of SCN-201 and SCN-203, and, although the melting curve of SCN-202 is not shown, it, in fact, lies between the curves for SCN-201 and SCN-203, as expected. The total spread in plateau temperatures among cells SCN-4, SCN-5, SCN-201, SCN-202, and SCN-203 is only about 0.001 °C.

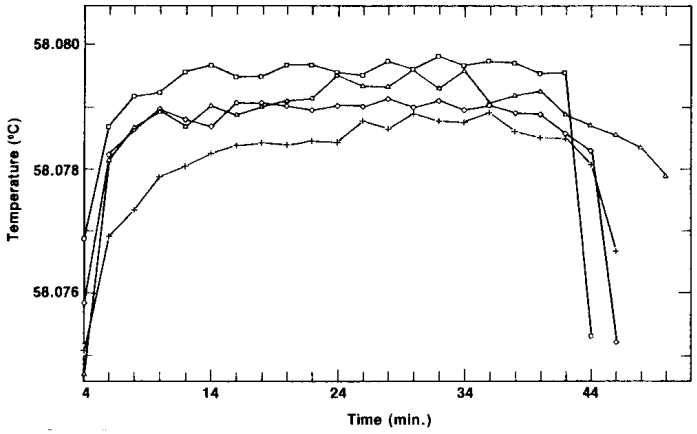

Fig. 3. Freezing curves of four samples of succinonitrile in a gradient of 2.31 °C

+ refers to SCN-1, □ to SCN-201, Δ to SCN-202, and ◇ to SCN-203

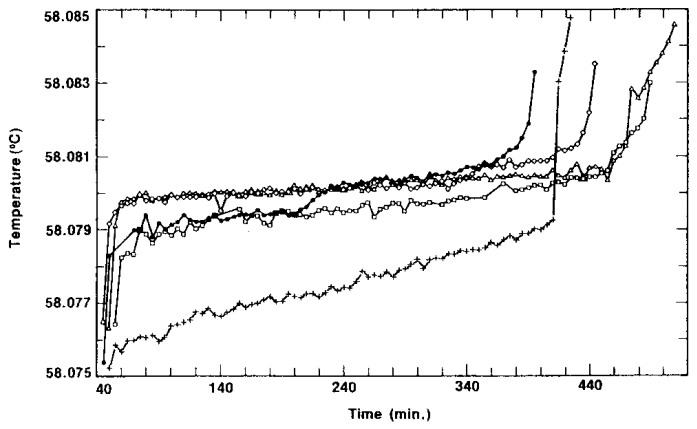

Fig. 4. Melting curves obtained for succinonitrile samples SCN-1 (+), SCN-4 (□), SCN-5 (△), SCN-201 (◇), and SCN-203 (●) in a 0.29 °C gradient

The value of a temperature reference point should be independent of the surroundings—i.e., bath, furnace, or cryostat—in which it is used. This was tested for the cells of SCN by measuring their temperature as a function of time during melting of the samples in baths at various temperatures. Because the latent heat of fusion for a given sample of material is a constant and the amount of heat transferred to the sample is directly proportional to the temperature difference, ΔT, between the sample and its surroundings, the length of time of coexistence of the solid and liquid phases (the length of time that a constant temperature environment is available for calibration) is inversely proportional to ΔT. Melting curves of SCN-201 obtained for several temperature differences are shown in Figure 5. It is seen that the various differences between the bath temperature and the plateau temperature had no effect except to change the duration of the plateau. This is good evidence that the thermometer wells of the small cells are sufficiently deep that the thermometer was indicating the temperature of the SCN and not sensing the external environment. If one desired to use thermometers other than thermistor thermometers with SCN cells, the length and diameter of the cells and of the thermometer wells could be adapted.

Table 2 gives the results of the determinations of the triple-point temperatures of SCN, corrected for hydrostatic-head effects, in the six small cells of this study. The correction for the hydrostatic head is calculated to be about 0.00016 °C (or, for our purposes, 0.0002 °C). Among the values listed in Table 2 are the temperatures at the liquidus, t_ℓ, and solidus, t_s, points determined by extrapolation of the plateaus of the melting curves, and the melting ranges for the various samples, i.e., the differences in temperature between the liquidus and the solidus points. Table 2 also lists the temperatures, t_m, at about the midpoints of the plateaus of the melting curves, i.e., at the points at which the fraction of SCN melted is about 0.5. These are the points most appropriate for calibration purposes.

The uncertainties in the temperatures were estimated as follows. The resolution of the thermistor thermometer system (*16*) was approximately ±0.0001 °C, and the overall statistical uncertainty in the temperatures measured with that system was estimated to be approximately ±0.00025 °C. There is a systematic uncertainty of approximately ±0.0006 °C relative to the IPTS-68, owing to the

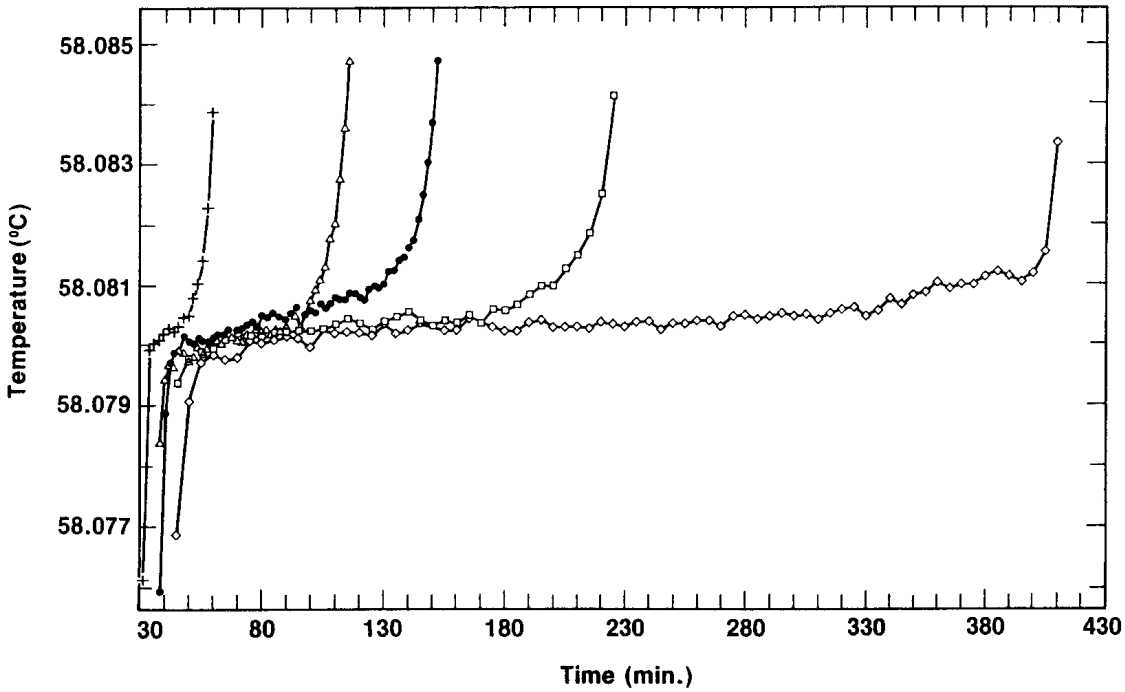

Fig. 5. Melting curves obtained for succinonitrile sample SCN-201 in several different gradients
+ represents data obtained in a gradient of 2.31 °C, Δ that with 1.16 °C, ● that with 0.87 °C, □ that with 0.58 °C, and ◇ that with 0.29 °C

Table 2. Transition Temperatures of Six Samples of Succinonitrile

°C			
t_l	t_s	t_m	$t_l - t_s$, m°C[a]
	SCN-1		
58.078_9	58.075_7	58.077_3	3.2
	SCN-4		
58.080_5	58.078_7	58.079_6	1.8
58.080_5	58.078_6	58.079_6	1.9
	SCN-5		
58.080_5	58.079_6	58.080_0	0.9
58.080_5	58.079_1	58.079_8	1.4
	SCN-201		
58.080_5	58.079_6	58.080_0	0.9
58.080_4	58.079_5	58.079_9	0.9
58.080_4	58.079_6	58.080_0	0.8
58.080_5	58.079_3	58.079_9	1.2
58.080_6	58.079_7	58.080_1	0.9
58.080_8	58.080_1	58.080_4	0.7
58.080_4	58.079_7	58.080_0	0.7
58.080_5	58.079_3	58.079_9	1.2
58.080_4	58.079_1	58.079_7	1.3
58.080_4	58.079_3	58.079_8	1.1
58.080_5	58.079_1	58.079_8	1.4
	SCN-202		
58.080_0	58.078_0	58.079_0	2.0
	SCN-203		
58.079_7	58.078_6	58.079_2	1.1

Mean of 15 measurements of t_l on SCN-4, SCN-5, and SCN-201 is 58.080_5 °C, with a standard deviation of the mean of 0.0001 °C.

Mean of 15 measurements of t_m on SCN-4, SCN-5, and SCN-201 is 58.079_9 °C, with a standard deviation of the mean of 0.0001 °C.

Mean of 11 measurements of t_m on SCN-201 is 58.080_0 °C, with a standard deviation of the mean of 0.0001 °C.

[a] 1 m°C = 0.001 °C.

calibration (*17*) of SPRTs and the fact that the platinum elements are "real" (as opposed to theoretically performing) materials (*5*). The errors due to differences in self-heating in the calibrations and in the experiments and those due to immersion of the thermistors are negligible. Thus, the total uncertainty in our determination of temperatures on the IPTS-68 with the thermistor thermometers was about ±0.001 °C. The error due to the presence of impurities in our purest SCN is estimated to be no greater than 0.0005 °C, so the total uncertainty in the triple-point temperature is estimated to be about ±0.0015 °C.

The average value of t_m for our purest samples of SCN was found to be 58.0799 °C, with an estimated uncertainty of ±0.0015 °C relative to the IPTS-68. As illustrated in Figures 2–5 and Table 2, the temperature of the liquid–solid transition varied slightly with the fraction of SCN melted, but the temperature of the transition, when starting initially at a point at which about half of the material was melted, changed by less than 0.001 °C over several hours when a cell was in an environment in which the temperature was approximately ±0.3 °C removed from t_m. As shown in Table 2, t_m was reproducible from day to day and from cell to cell to within about ±0.0003 °C (three standard deviations).

Discussion

The melting/freezing plateau, an approximation to the triple point, of succinonitrile is a good temperature reference point, suitable for use in many scientific disciplines. It could easily serve as the temperature against which to reference heat stability or denaturation studies of proteins and enzymes, and coagulation components in many areas of chemistry and immunology research, development, and service testing. The melting and freezing procedures of SCN are very straightforward and can be used very easily to quickly realize the plateau temperature at 58.080 ± 0.002 °C. Thus, the SCN fixed point could be as valuable as the gallium fixed point, the use and value of which have been described elsewhere (*1, 18, 19*).

The use of the SCN triple point[5] in conjunction with the gallium melting point and the water ice point or triple point will enable one to calibrate thermistors directly and accurately on the IPTS-68. From data to be published elsewhere

[5] Cells of SCN similar to the mini-cells used in this investigation will be available in a few months as SRM 1970 from the Office of Standard Reference Materials of the National Bureau of Standards.

at a later date, we have found that by determining the constants a, b, and c of the equation

$$1/T = a + b \log R + c (\log R)^3$$

from the data obtained at the three calibration points, temperatures (T) derived with this equation from the thermistor resistances (R) measured in the range from 0 to 70 °C are in agreement with those measured with an SPRT to within a total uncertainty of less than ±0.005 °C (three standard deviations). This uncertainty is at least sixfold less than is the case for the best liquid-in-glass thermometers at temperatures above 38 °C. For total-immersion liquid-in-glass thermometers (which require a large volume of material for any temperature measurement), the smallest uncertainty of an NBS calibration is ±0.03 °C. The uncertainty for a partial-immersion thermometer, at temperatures outside the range 24 to 38 °C, is ±0.3 °C. Thus, by using the SCN fixed point to calibrate thermistor thermometers, accuracy of temperature measurements can be much improved.

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